

CLAIMS:

1. A method for producing a sense RNA molecule, comprising:

providing a single stranded cDNA molecule having 5' and
5 3' ends;

attaching an oligodeoxynucleotide tail to the 3' end of said single stranded cDNA molecule;

providing a double stranded RNA polymerase promoter having a sense strand and antisense strand, wherein the
10 sense strand comprises a single stranded 3' overhang comprising a sequence complementary to said oligodeoxynucleotide tail;

annealing said double stranded RNA polymerase promoter to said oligodeoxynucleotide tail by complementary base
15 pairing with said 3' overhang sequence;

ligating the 5' end of the antisense strand of said double stranded RNA polymerase promoter to the 3' end of said oligodeoxynucleotide tail; and

initiating RNA transcription using an RNA polymerase
20 which recognizes said double stranded promoter, thus producing a sense RNA molecule.

2. The method of claim 1, wherein a) comprises providing a mRNA transcript having 5' and 3' ends; and synthesizing a single stranded cDNA molecule from said mRNA
25 transcript.

3. The method of claim 2, wherein synthesis of the single stranded cDNA molecule comprises reacting the mRNA molecule with a RNase H⁻ reverse transcriptase.

4. The method of claim 2, wherein synthesis of the
30 single stranded cDNA molecule comprises reacting the mRNA molecule with an oligodT primer.

5. The method of claim 2, wherein synthesis of the single stranded cDNA molecule comprises reacting the mRNA molecule with a random primer.

5 6. The method of claim 2, further comprising purifying the single stranded cDNA molecule prior to attaching the oligodeoxynucleotide tail.

7. The method of claim 6, further comprising degrading the mRNA transcript prior to purifying the single stranded cDNA molecule.

10 8. The method of claim 6, wherein the mRNA transcript is not degraded prior to purifying the single stranded cDNA molecule.

9. The method of claim 1, wherein the oligodeoxynucleotide tail is a homopolymeric tail.

15 10. The method of claim 9, wherein the homopolymeric tail is a polydT tail.

11. The method of claim 1, wherein the oligodeoxynucleotide tail is attached to the 3' end of the single stranded cDNA molecule using terminal deoxynucleotidyl transferase.

12. The method of claim 1 or 2, wherein the double stranded RNA polymerase promoter is a T7, T3, or SP6 promoter.

13. The method of claim 12, wherein the double stranded RNA polymerase promoter is a T7 promoter.

14. The method of claim 1, wherein the single stranded 3' overhang comprises a sequence of adenosine bases.

15. The method of claim 1, wherein ligation is performed using T4 DNA ligase.

30 16. The method of claim 1, wherein RNA transcription is initiated using T7 RNA polymerase.

17. The method of claim 1, further comprising synthesizing second strand cDNA prior to initiating RNA transcription.

5 18. The method of claim 17, wherein the second strand cDNA is synthesized using DNA polymerase.

19. The method of claim 17, wherein the second strand cDNA is synthesized by extension of the 3' overhang of the sense strand of the RNA polymerase promoter.

10 20. The method of claim 17, wherein the second strand cDNA is synthesized using a random primer, thus producing random-primed second strand cDNA fragments.

21. The method of claim 20, wherein the random-primed second strand cDNA fragments are ligated together prior to initiating RNA transcription.

15 22. The method of claim 1, further comprising amplifying the resulting sRNA molecule.

23. The method of claim 22, wherein the sRNA amplification is initiated using a combination of oligodT and random primers.

20 24. The method of claim 1, wherein the resulting sRNA molecule comprises a polyA tail.

25. The method of claim 24, wherein the polyA tail is attached using polyA polymerase.

25 26. The method of claim 1, further comprising reverse transcribing the resulting sRNA molecule, thereby producing a single stranded cDNA molecule.

27. The method of claim 26, wherein the reverse transcription comprises incorporating detectably labeled nucleotides into the single stranded cDNA molecule.

30 28. The method of claim 27, wherein the detectably labeled nucleotides comprise a fluorescent dye.

29. The method of claim 28, wherein the fluorescent dye is cy3 or cy5.

30. The method of claim 26, further comprising attaching at least one detectable label to the resulting
5 cDNA molecule.

31. A method for probing a nucleic acid microarray, comprising contacting a nucleic acid microarray with the detectably labeled cDNA of claim 27, 28, 29, or 30.

32. The method of claim 2, wherein the mRNA transcript
10 is of mammalian origin.

33. The method of claim 2, wherein the mRNA transcript is of human origin.

34. The method of claim 2, wherein the mRNA transcript is isolated from a biological source comprising degraded
15 RNA.

35. A kit for producing at least one sRNA molecule, comprising: a double stranded RNA polymerase promoter having a sense strand and antisense strand, wherein the sense strand of said double stranded RNA polymerase promoter
20 comprises a single stranded 3' overhang sequence; and instructional materials for generating sRNA molecules using said double stranded promoter.

36. The kit of claim 35, further comprising at least one enzyme for attaching an oligodeoxynucleotide tail onto
25 the 3' end of a single stranded cDNA molecule, wherein the oligodeoxynucleotide tail is complementary to the single stranded 3' overhang sequence of said double stranded RNA polymerase promoter; and at least one enzyme for ligating said double stranded promoter onto the 3' end of said cDNA
30 molecule.

37. The kit of claim 36, further comprising terminal deoxynucleotidyl transferase and T4 DNA ligase.

38. The kit of claim 37, further comprising an oligodT primer; a random primer; dNTPs; and a RNase inhibitor.

39. The kit of claim 38, further comprising a DNA polymerase.